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Ciliates in Rapid Gravity Filters of Waterworks Exploiting Deep Groundwaters

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KEY WORDS Ciliophora, Drinking water, Protozoa, Saprobic system

ABSTRACT Potable water is increasingly produced from deep (>100 m) tertiary groundwaters which often are completely reduced and contain high amounts of ammonium, methane, and hydrogen sulphide. They thus require special treatment which includes oxygenation and removal of the reduced contaminants by the biofilm developing in rapid gravity filters. The biofilm is heavily colonized with ciliates and microinvertebrates. A total of 38 species of ciliates was found in 42 samples taken from 4 waterworks in Germany during a period of 2 years. Only six species occurred in high numbers and in more than half of the samples: *Acineria uncinata, Aspidisca lynceus, Cinetochilum margaritaceum, Colpidium colpoda, Glaucoma scintillans,* and *Holosticha pullaster*. Five to thirteen species occurred per sample, and up to 6,000 individuals ml^{-1} biofilm were counted. There was a considerable fluctuation in the number of species and individuals, which could not be related to specific process parameters. The number of species and individuals decreased markedly from the filter surface to its centre. Colonization of the filters very likely occurs randomly via impure surface waters. The ciliate communities found consist mainly of alphamesosaprobic to polysaprobic species and thus closely resemble those known from activated-sludge processes. This is explained by the specific conditions near and in the biofilm, which is probably microaerobic and highly productive, providing microaerobic bacterial feeders with copious food. Obviously, it is the microenvironment which determines the occurrence of certain species. Thus, future research on the autecology of the indicator species used in the saprobic system should concentrate on their microenvironments.

INTRODUCTION

It is increasingly difficult to find surface and groundwaters suitable for drinking purposes. Even groundwater is often contaminated with nitrates, pesticides, and other potentially toxic substances. There is thus an increasing tendency to exploit deep, better protected groundwaters (Gierig, 1993). However, such waters, although almost pristine, are usually completely reduced and contain not only high amounts of iron and manganese but also hydrogen sulphide (H_2S), methane (CH_4), and ammonium (NH_4^+). Special technologies have been developed to remove these inorganics and organics to produce water fit for human consumption. One of these methods is oxygenation followed by rapid gravity filtration through fine-grained gravel. In these reactors a biofilm consisting of bacteria, protozoa, and microinvertebrates develops and oxidizes the reduced organic substances.

The occurrence of protozoa in pristine groundwater is still insufficiently documented. In fact, there are only a dozen papers which reliably report protozoa in groundwater, and these studies are confined principally to a few aquifers in North America and Europe (Hirsch and Rades-Rohkohl, 1983; Kinner et al., 1991; Lüpkes, 1974; Novarino et al., in press; Sinclair, 1991). There are, however, many data on protozoa in wells, waterworks, and public water supplies (for reviews see Curds, 1992; Kustermann, 1958; Liebmann, 1958; Playfair, 1913; Ruttner, 1906; Walton, 1930). In Czechoslovakia up to 20 organisms ml^{-1} are allowed in public water supplies and up to 100 heterotrophic flagellates and 10 ciliates ml^{-1} in local water supplies (Moravcova, 1986). Hazards posed by certain protozoan parasites are being increasingly recognized. In the USA, for instance, 92 cases of waterborne giardiasis have been registered (Smith and Smith, 1990).

A pilot investigation of a water treatment plant using tertiary groundwater from 150 m depth showed the biofilm of the oxidators and filters to be colonized with a huge number of ciliates and microinvertebrates which were sometimes washed into the underground pipes and caused aesthetic problems (Gierig, 1993). A closer examination of this community was thus called for, especially since few detailed investigations on the protozoan fauna of potable water treatment works are available (Curds, 1992). Furthermore, such investigations could provide information on the little known groundwater ciliates (Lüpkes, 1974).

MATERIALS AND METHODS

Study Sites and Processing of the Raw Water

Most investigations were undertaken at the water treatment plant of Straubing, a small town 100 km

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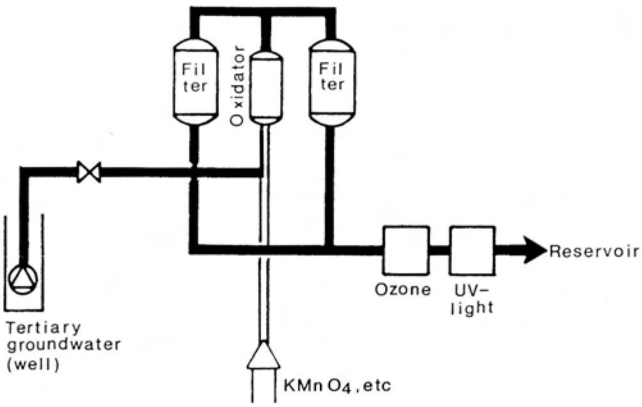


Fig. 1. Diagram of tertiary water treatment at Straubing and Straβkirchen. (Adapted from Stadtwerke Straubing, 1986.)

northeast of Munich, Bavaria (Germany). Some comparative studies were done in the waterworks of towns near Straubing, viz. in Ruhstorf, Straβkirchen, and Bad Füssing which were, however, inoculated with material (sludge) from Straubing. In Ruhstorf and Bad Füssing the tertiary water is mixed with quaternary groundwater from ordinary wells.

A detailed description of the waterworks is found in Gierig (1993) and in Stadtwerke Straubing (1989). I thus provide only an abstract from these data, amended with a scheme of the treatment process (Fig. 1) and a chemical analysis of the raw water (Table 1), which is about 30,000 years old and pumped from tertiary quartz gravel located in a depth of about 150 m. The raw water is anoxic and contains hydrogen sulphide, methane, and ammonium. Thus, it is first aerated with compressed air while potassium permanganate ($KMnO_4$) and potassium phosphate (K_2HPO_4) are admixed in the "oxidator" to adjust the redox potential near to 0 mV and to provide nutrients for bacteria. From the oxidator the water flows to the rapid gravity filters (bioreactors), where the reduced contaminants are oxidized by the bacteria of the biofilm. Both oxidators and filters are 3.5 m high by 4 m in diameter. They are filled with 1–2 mm grained quartz gravel and are closed (i.e., have no contact with the environment). The filter rate is about 5 m h^{-1}, corresponding to an average retention time of about 25 min in the filter. The oxidized water is cleared by a steel filter (25 μm) and disinfected with ozone and UV light (Fig. 1). To remove the excess sludge (biofilm), the oxidators and filters are backwashed if the differential pressure exceeds 0.3 bar.

TABLE 1. Characteristics of the raw water (From Gierig, 1993)

Parameter	Amount
Temperature (°C)	14.6
Redox potential (mV)	−354
pH	7.7
Oxygen (O_2, mg/l)	<0.1
Ammonium (NH_4^+, mg/l)	0.7
Nitrate (NO_{3^-}, mg/l)	<0.1
Nitrite (NO_{2^-}, mg/l)	<0.005
Phosphate (PO_{3^-}, mg/l)	0.02
Organic carbon (TOC, mg/l)	3.8
Hydrogen sulphide (H_2S, mg/l)	0.2
Methane (CH_4, mg/l)	3.5

TABLE 2. Origin and designation of samples

Waterworks	Sample number: Oxidator	Sample number: Filter
Straubing	1, 2, 5–7, 21–23, 27–29, 39	3, 4, 8–12, 24–26, 30–32, 40–42
Ruhstorf	13, 33	14–16, 34
Straβkirchen	17, 35	18, 36
Bad Füssing		19, 20, 37, 38

Sampling and Counting Procedures

Forty-two samples were taken from the oxidators and filters during a period of 2 years, viz. between 29 March 1990 and 12 March 1992 (Table 2). Usually, the detached biofilm contained in the backwash water was investigated. This material looks very similar to activated sludge from ordinary sewage plants. Three random subsamples of 0.05 ml each were investigated from all samples. The species seen during an investigation period of about 30 min were noted, and their individual abundances were estimated with a rating scale: 0 = not found, 1 = very sparse, 2 = sparse, 3 = sparse to medium, 5 = medium, 7 = numerous, 9 = very numerous.

More exact quantitative data were obtained on some occasions by counting the ciliates in 100 μl sludge (biofilm) detached from the filter material by shaking a certain amount of it for a few minutes. To transform the values to 1 l quartz gravel, the amount of sludge was determined with the Imhoff funnel.

Determination of Species, Nomenclature

All species were determined from life using interference contrast optics and recently published monographs on the ciliates of the saprobic system (Foissner et al., 1991, 1992, 1994, 1995). Nomenclature is also according to these reviews. Methods used to prepare Figures 2–15 are described in Foissner (1991).

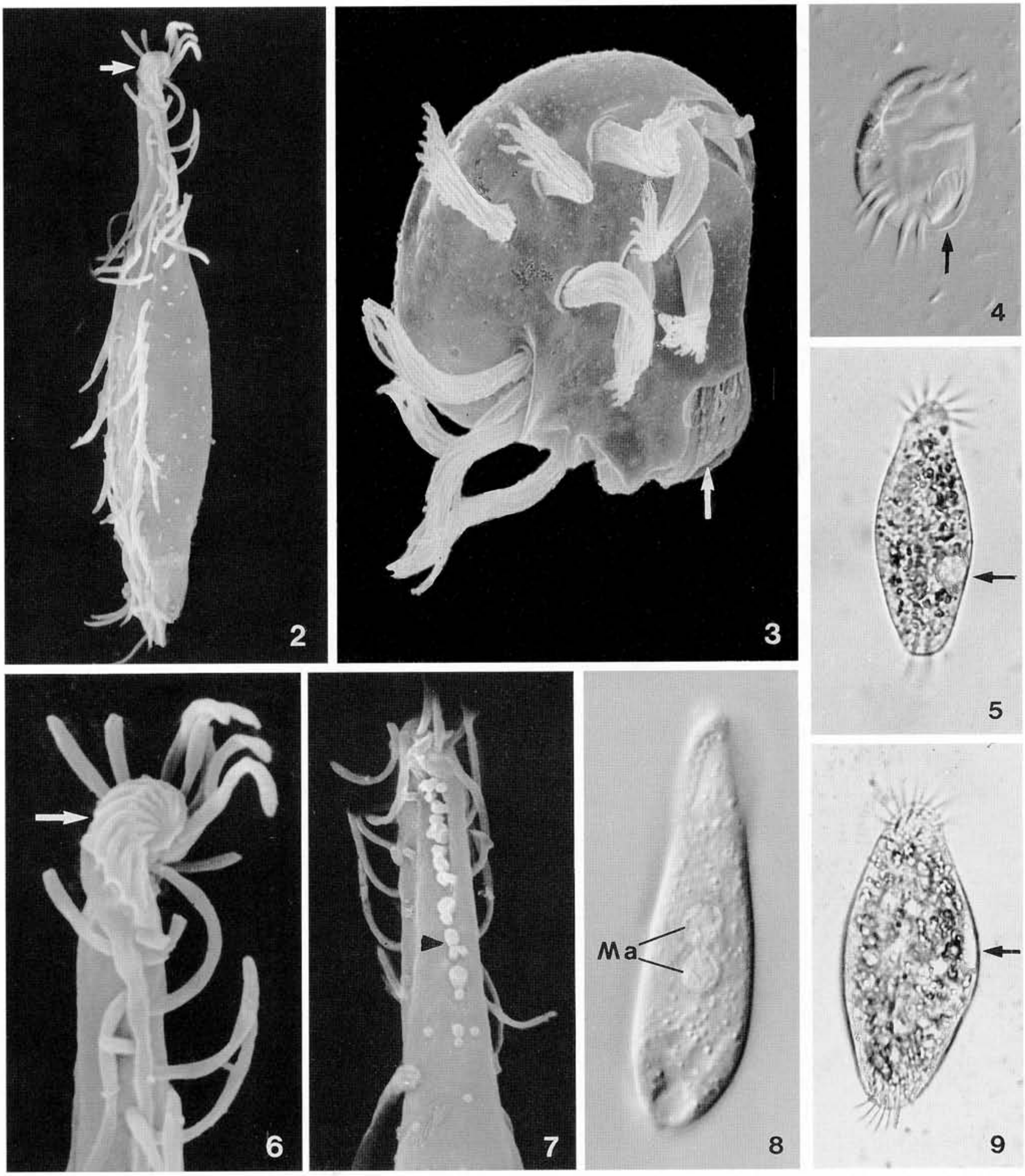
Similarity Analysis

Similarity between the ciliate communities in each plant and on each sampling date was calculated with the indices suggested by Bray and Curtis (1957) and Jaccard (1902). The similarity values obtained were summarized by clustering using the UPGMA (unweighted group mean, average distance criteria; Sneath and Sokal, 1973) algorithms of the CLUSTAN program.

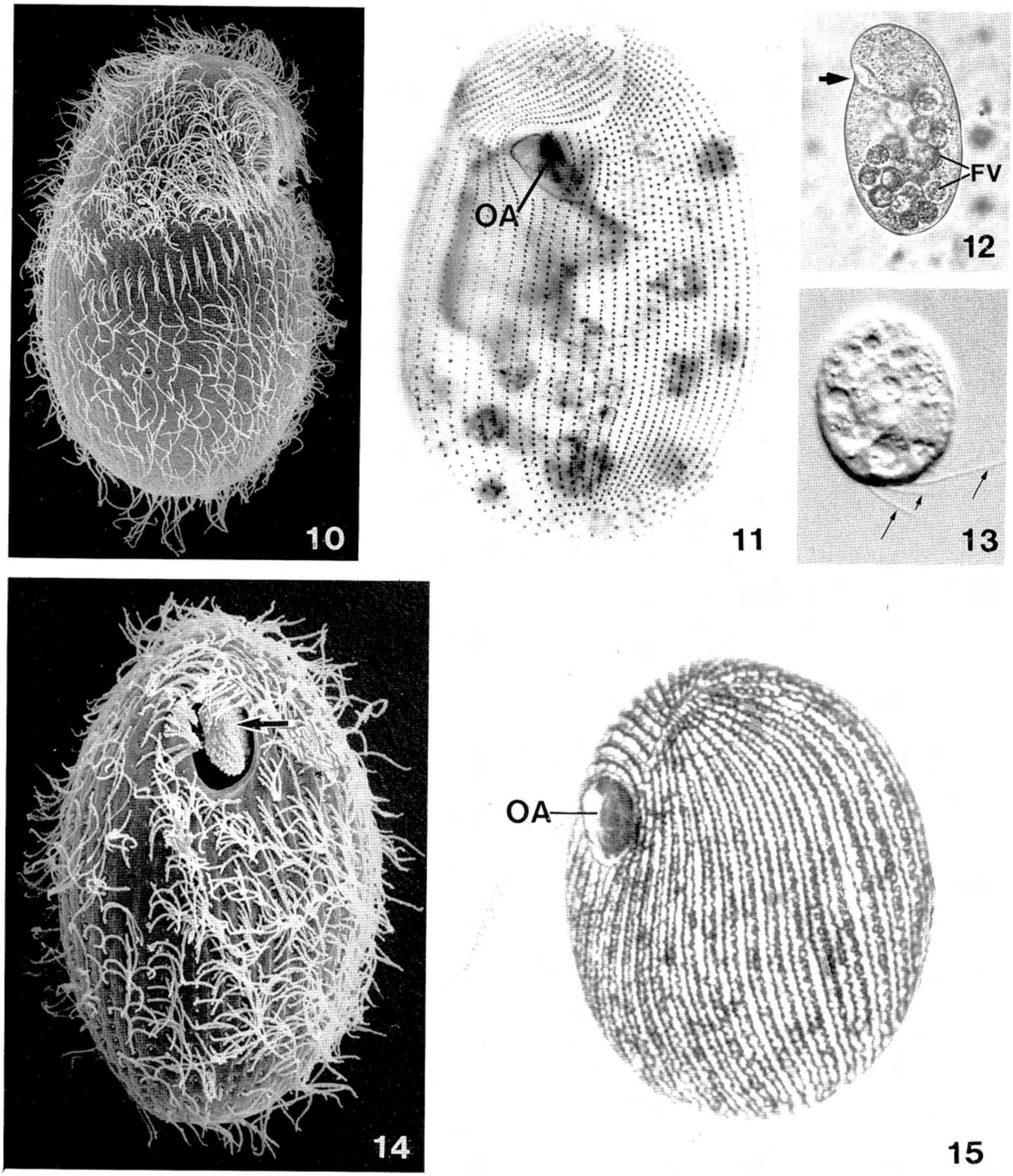
RESULTS

Comparison of the Ciliate Communities in Waterworks, Oxidators, and Filters

A total of 38 ciliate taxa was found in the 42 samples investigated. Fourteen species were recorded only in



Figs. 2–9. Common ciliates in rapid gravity filters as seen in the scanning electron microscope (2, 3, 6, 7) and light microscope (4, 5, 8, 9). Figs. 2, 6–8: *Acineria uncinata* is a small (length 40 μm), pleurostomatid predator with a characteristic oral ornamentation (arrow in Figs. 2, 6) and a row of specialized cilia (arrowhead in Fig. 7) at the dorsal anterior end. It has two macronuclear nodules (Ma in Fig. 8), a major difference relative to the mononucleate *Litonotus alpestris*. Figs. 3, 4: *Aspidisca lynceus* is a small (45 μm long), hypotrichous ciliate which feeds on bacteria collected by the adoral membranelles (arrows) located in a deep buccal cavity. The cilia are grouped in thick bundles (cirri). Figs. 5, 9: *Holosticha pullaster* and *Tachysoma pellionellum* are medium-sized (length 60–100 μm), omnivorous hypotrich ciliates which look very much alike but differ distinctly in the location of the contractile vacuole (arrows).



Figs. 10–15. Common bacterivorous tetrahymenid ciliates in rapid gravity filters as seen in the scanning electron microscope (10, 14) and light microscope (11–13, 15). Figs. 11 and 15 are from silver impregnated specimens and show the arrangement of the somatic cilia and the location of the oral apparatus (OA). Figs. 10–12: *Colpidium colpoda* is a polysaprobic species, about 150 μm long, which collects bacteria in the small oral funnel (arrow in Fig. 12, OA in Fig. 11) and digests them in compact food vacuoles (FV in Fig. 12). Fig. 13: *Cinetochilum margaritaceum* is a tiny (20–30 μm), eurysaprobic species having long caudal cilia (arrows). Figs. 14, 15: *Glaucoma scintillans* is a polysaprobic to alpha-mesosaprobic species, about 60 μm long, which collects bacteria with its "heavy" adoral membranelles (arrow in Fig. 14, OA in Fig. 15).

TABLE 3. Ecological characteristics of ciliates in 42 samples of backwash sludge from oxidators and filters of four waterworks in Germany

Taxa	Occurrence[1]	Feeding type[2]	Saprobity[3]	Presence[4] (%)	Maximum abundance[5]	Cysts[6]
Acineria incurvata DUJARDIN, 1841	S	P	p	2.4	single record, 2	+
Acineria uncinata TUCOLESCO, 1962	S, R, K, F	P	a-p	85.7	5	+
Aspidisca cicada (MÜLLER, 1786)	K	B	a-b	4.8	3	?
Aspidisca lynceus (MÜLLER, 1773)	S, R, K, F	B	b-a	78.6	5	?
Calyptotricha lanuginosa (PENARD, 1922)	K, F	B	a	7.1	1	?
Chilodonella uncinata (EHRENBERG, 1838)	S, R, F	B	a	42.9	2	+
Chilodonellidae Gen. sp.	S, F	B	?	7.1	2	?
Chilodontopsis depressa (PERTY, 1852)	S	D	b	19.0	2	?
Chlamydonella rostrata (VUXANOVICI, 1963)	K	D	?	7.1	1	?
Cinetochilum margaritaceum (EHRENBERG, 1831)	S, R, K, F	B	o-p	93.0	5	+
Colpidium colpoda (LOSANA, 1829)	S, R, F	B	p	57.1	5	−
Cyclidium glaucoma MÜLLER, 1773	S	B	a	11.9	2	+
Dexiostoma campylum (STOKES, 1886)	F	B	p	2.4	single record, 1	−
Dexiotricha tranquillus (KAHL, 1926)	S, K	B	?	35.7	3	?
Euplotes affinis (DUJARDIN, 1841)	R	B	b-a	2.4	single record, 1	?
Fuscheria lacustris SONG & WILBERT, 1989	K	P	?	2.4	single record, 1	?
Glaucoma scintillans EHRENBERG, 1830	S, R, F	B	p-a	54.8	5	−
Holosticha monilata KAHL, 1928	K	O	a-b	7.1	1	+
Holosticha pullaster (MÜLLER, 1773)	S, R, K, F	O	b-a	93.0	3	?
Lacrymaria olor (MÜLLER, 1786)	K	P	b	2.4	single record, 1	+
Litonotus alpestris FOISSNER, 1978	S	P	b-a	7.1	2	?
Litonotus trichocystiferus FOISSNER, 1984	F	P	?	4.8	1	?
Litonotus varsaviensis WRZEŚNIOWSKI, 1866	F	P	b-a	7.1	1	?
Loxophyllum utriculariae (PENARD, 1922)	S, F	P	b	35.7	2	?
Microthorax sp.	S	B	?	2.4	single record, 1	?
Odontochlamys alpestris FOISSNER, 1981	F	B	b-a	4.8	2	+
Orthotrochilia sp.	S, R, F	?	?	21.4	2	?
Podophrya fixa (MÜLLER, 1786)	K	P	a	2.4	single record, 1	+
Pseudochilodonopsis fluviatilis FOISSNER, 1988	S, K, F	D	b-a	16.7	1	?
Sterkiella histriomuscorum (FOISSNER et al., 1991)	R	O	a	2.4	single record, 1	+
Tachysoma pellionellum (MÜLLER, 1773)	S	B	b-a	4.8	2	?
Thigmogaster oppositevacuolatus AUGUSTIN & FOISSNER, 1989	K	B	a-b	4.8	1	?
Tokophrya infusionum (STEIN, 1859)	R	P	b-a	7.1	2	+
Trithigmostoma cucullulus (MÜLLER, 1786)	S	O	a-p	2.4	single record, 1	−
Vorticella aquadulcis-complex	S, K, F	B	b-a	23.8	2	+
Vorticella convallaria-complex	S, R, K, F	B	a	26.2	1	+
Vorticella infusionum-complex	S, R, F	B	p-a	28.6	3	+
Vorticella platysoma STOKES, 1887	S	B	?	11.9	2	?

[1]Waterworks Straubing (S), Straβkirchen (K), Bad Füssing (F), and Ruhstorf (R).
[2]According to Foissner et al. (1991, 1992, 1994, 1995). Only main food sources are given. B = bacterivorous, D = diatoms, O = omnivorous, P = predator (other ciliates).
[3]Saprobic assessment according to Foissner et al. (1995): o = oligosaprobic, b = beta-mesosaprobic, a = alpha-mesosaprobic, p = polysaprobic.
[4]Percentage of samples in which the species occurred (total = 42 samples).
[5]Rated according to the scale given in Materials and Methods.
[6]+, known, − very likely absent, ? not known.

one or two samples, and merely six species occurred in at least half of the samples (Table 3): *Acineria uncinata,* a small (~40 μm) pleurostomatid predator (Figs. 2, 6–8); *Aspidisca lynceus* and *Holosticha pullaster,* small to medium-sized (~80 μm) bacterivorous hypotrichs (Figs. 3–5); and *Cinetochilum margaritaceum, Glaucoma scintillans,* and *Colpidium colpoda,* small to medium-sized bacterivorous tetrahymenids (Figs. 10–15). There were 5–13, on average 8.3, species per sample.

No distinct differences could be found among the ciliate communities of the four waterworks (Fig. 16). This result was not too surprising, because all were inoculated with the biofilm from the Straubing waterworks. However, a closer examination of Figure 16 revealed some distinctions, viz. the samples from Straβkirchen (nos. 17, 18, 35, 36) formed a subcluster due to a rather high proportion of species (*Aspidisca cicada, Chlamydonella rostrata, Fuscheria lacustris, Holosticha monilata, Lacrymaria olor, Podophrya fixa, Thigmogaster oppositevacuolatus*) restricted to this plant. Six species (*Dexiostoma campylum, Euplotes affinis, Litonotus trichocystiferus, Odontochlamys alpestris, Sterkiella histriomuscorum, Tokophrya infusionum*) occurred only in the waterworks of Ruhstorf and Bad Füssing, where quaternary groundwater is admixed. These samples (nos. 13–16, 33, 34 and 19, 20, 37, 38) were randomly distributed within the main cluster. When abundance estimates were included in the similarity analysis (Bray and Curtis index), the resulting cluster was very similar to that of species only (Jaccard index) and for the sake of brevity is not shown here.

The dendrogram (Fig. 16) also was examined to detect differences between the ciliate communities of oxidators and filters. No evidence of segregation was found.

Abundances and Vertical Distribution

The number of ciliates was high in most samples, viz. up to 6,000 cells ml^{-1} biofilm (Tables 4, 6). Usually, ciliates were more numerous in the oxidators than in the filters, and one to three species accounted for the majority of the specimens.

There was a considerable fluctuation in the number of ciliate species and individuals (Table 5), which could not be related to specific process parameters. The sudden, massive appearance (invasion?) of *Colpidium colpoda* between 29 March 1990 and 9 October 1990 was especially remarkable, because it was coupled with a total disappearance of *Glaucoma scintillans,* which was eudominant on 29 March 1990, when the investigation commenced. Both species prefer polysaprobic conditions and often occur together (Foissner et al., 1994). Thus, their different behaviour in the waterworks is difficult to explain. However, food competition could have played some role because both feed on bacteria.

There was a distinct decrease of species and individuals from the filter surface to its center, whereas the species composition hardly changed (Tables 6, 7). This decrease correlated with an apparent thinning of the biofilm and a distinct decrease in the number of free bacteria.

Correlations Between Ciliates and Ammonium (NH_4^+)

On 13 sampling occasions ciliates and NH_4^+ concentrations were simultaneously recorded. These pairs were investigated with Spearman's ranked correlation coefficient using species number, total ranked abundances, and the ranked abundances of the eight most frequently occurring species (*Acineria uncinata, Aspidisca lynceus, Chilodonella uncinata, Cinetochilum margaritaceum, Colpidium colpoda, Glaucoma scintillans, Holosticha pullaster, Vorticella infusionum*). The correlation coefficients obtained varied between −0.365 (*C. margaritaceum:* NH_4^+) and 0.360 (*V. infusionum:* NH_4^+)—that is, all were statistically insignificant ($P \geq 0.1$).

DISCUSSION

On the Origin of the Ciliate Fauna in Oxidators and Filters

Although it was not the principal objective of this study to discover groundwater specific ciliates, I hoped to find some, at least in the samples from the waterworks of Ruhstorf and Bad Füssing, where aerobic quaternary groundwater is admixed with anoxic tertiary water. This expectation was not unfounded, because there are several reports on protozoa in groundwater aquifers and some multicellular, true groundwater organisms often enrich in slow sand filters, very likely due to increased food supply (Husmann, 1978). Hirsch and Rades-Rohkohl (1983) observed 10 protozoan species (amoebae, flagellates, ciliates) in groundwater pumped upward from 10 m depth (7 m below the water table). Lüpkes (1974) described three new peritrichs from a 3 m deep groundwater aquifer in Germany, and various heterotrophic flagellates and naked amoebae, but no ciliates, have been reported from pristine groundwaters in the USA, down to a depth of 436 m (Kinner et al., 1991; Novarino et al., in press; Sinclair, 1991; Sinclair and Ghiorse, 1987).

However, all ciliate species found during the present investigation are common inhabitants of surface waters, including the three taxa not determined to species level, but which I know from several streams and rivers. Likewise, no stygophilic metazoans were found in the oxidators and filters, although such organisms (e.g., *Cavernocypris subterranea*) occurred in the reservoirs where the purified tertiary water is stored with ordinary quaternary groundwater (Gierig, 1993). Furthermore, few additional species occurred in the waterworks of Ruhstorf and Bad Füssing, where the tertiary groundwater is mixed with quaternary water. It is thus likely that the oxidators and filters were colonized randomly via contaminated filter material and/or transient contact of the groundwater with impure surface water. This interpretation is supported by the occurrence of some ciliates which are unable to produce viable seeds (resting cysts), especially *Colpidium colpoda* and *Glaucoma scintillans* (Foissner et al., 1994). At least these species must have entered the purification system as active stages (i.e., via a "wet way"). In fact, while this paper was in press, the plant operators con-

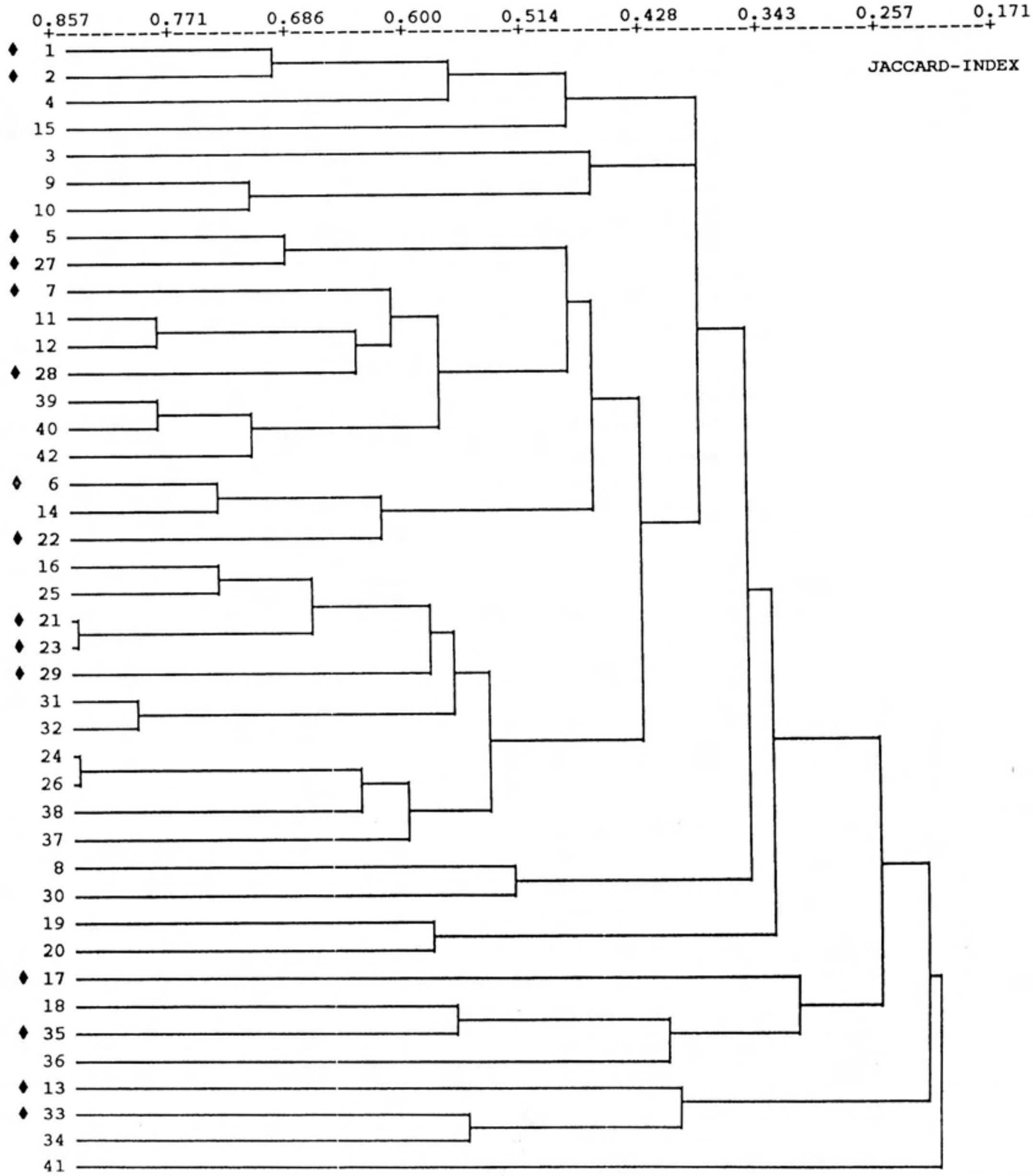


Fig. 16. Clusters of Jaccard similarity indices for the ciliate communities in oxidators (♦) and filters (without symbol) of four waterworks (see Table 2 for numbering and origin of samples).

fessed that a mixture of tertiary and quaternary groundwater was used several times for filter backwash in the Straubing waterworks, too! Thus, the ciliates entered the "closed" system obviously via the quaternary water.

The lack of true groundwater organisms in the oxidators and filters, even if quaternary groundwater is admixed, may be caused by the special chemical (rather high content of ammonium) and physical (high shearing forces during backwash) properties of the raw water. It is well known that true groundwater organisms are very sensitive to any alteration of their environment and cannot survive for longer periods in surface waters. Furthermore, subsurface bacterial populations may be inadequate to support ciliates. Subsurface bacterial numbers are often two to three orders of magnitude lower than those of surface soils, and ciliates have greater energy requirements than flagellates or amoebae (Fenchel, 1987). Another reason why groundwater ciliates were not present in oxidators or filters may have been that the groundwater was a highly reduced environment with H_2S and CH_4 present, whereas the oxidators and filters were aerobic environments. Ciliates able to live in highly reduced

TABLE 4. Abundances of dominant species in 1 l oxidator and filter gravel (From Gierig, 1993)[1]

	9 October 1990		29 January 1991	
Species	Oxidator	Filter	Oxidator	Filter
Aspidisca lynceus	25,000	7,200	20,250	5,700
Colpidium colpoda	77,500	15,000	12,250	0
Holosticha pullaster	16,000	2,400	33,000	3,600
Total number ml^{-1} sludge[2]	5,925	4,100	3,275	1,550

[1]Values are means of three replicates.
[2]Calculated from the total sludge contained in 1 l of gravel: 20 ml in the oxidator and 6 ml in the filter.

TABLE 5. Rated abundance fluctuations (See Materials and Methods) of the ciliates in oxidator 1 (Ox) and filter 1 (Fi) of the Straubing waterworks

	29 March 1990		9 October 1990		29 January 1990		18 June 1991	
Species	Ox	Fi	Ox	Fi	Ox	Fi	Ox	Fi
Acineria uncinata	1	3	5	0	2	2	2	1
Aspidisca lynceus	0	1	3	2	2	2	2	1
Chilodontopsis depressa	0	1	0	1	0	0	0	1
Cinetochilum margaritaceum	1	3	3	3	3	2	3	2
Colpidium colpoda	0	0	5	3	1	0	2	0
Cyclidium glaucoma	0	0	2	1	0	0	2	1
Dexiotricha tranquillus	1	0	2	0	1	0	1	0
Glaucoma scintillans	5	3	0	0	0	0	0	0
Holosticha pullaster	1	1	2	2	3	1	3	1
Litonotus alpestris	0	1	2	0	0	0	0	0
Loxophyllum utriculariae	0	0	0	1	0	1	0	1
Pseudochilodonopsis fluviatilis	0	1	0	0	0	1	0	1
Tachysoma pellionellum	0	1	0	0	0	0	0	0
Vorticella aquadulcis	1	0	0	0	0	0	0	0
Vorticella platysoma	0	0	2	0	0	0	0	0
Vorticella infusionum	0	0	0	0	1	1	0	0
Chilodonella uncinata	0	0	0	0	0	0	0	1
Microthorax sp.	0	0	0	0	0	0	0	1
Orthotrochilia sp.	0	0	0	0	0	0	1	1
Total species number	6	9	9	7	7	7	8	11

TABLE 6. Vertical distribution of dominant ciliate species in 1 l of filter gravel (From Gierig, 1993)[1]

	Depth in Filter					
Species	Surface	25 cm	50 cm	100 cm	150 cm	200 cm
Aspidisca lynceus	49,500	25,200	13,100	2,700	200	100
Cinetochilum margaritaceum	60,400	34,700	12,300	3,500	360	75
Holosticha pullaster	24,500	11,900	6,000	1,300	130	40

[1]Values are means of three replicates.

environments may be killed or at least be at a competitive disadvantage in an aerobic environment.

Structure of the Ciliate Community

The ciliate communities reported here are remarkable in several respects: they contain few species but many individuals and have few specific inhabitants. Thus, they strongly resemble the ciliate communities found in the biofilm of percolating filters and in activated sludge, except that these communities often possess a higher abundance of sessile peritrichs (Curds, 1992; Foissner et al., 1995; Madoni, 1991). Peritrichs are also dominant in slow sand filters (Curds, 1979; Lloyd, 1973) and sometimes become numerous in public water supplies (Ruttner, 1906). In the present study, their rather weak development might be associated with the high shearing forces occurring when the oxidators and filters are backwashed. It is well known that most peritrichs prefer low current velocities and are rare in highly turbulent activated sludge plants (Foissner et al., 1992). The counted and rated abundances (Tables 3–6) support the view that the biofilm of the oxidators and filters is similar to the sludge in percolating filters and activated plants, where abundances around 5,000 cells ml^{-1} are also common (Aescht and Foissner, 1992; Augustin et al., 1989; Liebmann, 1968; Madoni, 1991). Using a culture method and MPN counts, Richards (1974) found 10–150 ciliates (active + cystic?) per cubic centimeter of top sand, which nicely matches the numbers obtained using a direct counting method in the present study (<10 to 80 individuals per cubic centimeter of gravel; Tables 4, 6). Also as seen in this study, Richards (1974) found that numbers of ciliates declined sharply with depth (cf. Tables 6, 7).

The ciliate communities in oxidators and filters of

TABLE 7. Vertical distribution of ciliates in three filters of the waterworks in Straubing on 12 March 1992[1]

	Depth (cm) in filter 1						Depth (cm) in filter 2					Depth (cm) in filter 6				
Species	0	25	50	100	150	200	0	25	50	100	150	0	25	50	100	150
Acineria incurvata													2			
Acineria uncinata	1	1	1	1	1	1	1	1	1			3		2	1	1
Aspidisca lynceus	1	1	1	1	1	1	1	1				2	1	1		
Chilodonella uncinata		1		1		1	1					1	1			
Cinetochilum margaritaceum	1	1	1	1	1	1	2			1	1	3	2	1	1	
Colpidium colpoda					1			1				2	2	1	1	
Dexiotricha tranquillus	1							1								
Glaucoma scintillans							2						1			
Holosticha pullaster	1	1	1	1	1	1	2	1	1	1	1	2	1	1	1	1
Vorticella aquadulcis	2	1	2	2	1	1	1	1	1	1		1	1	1	1	1
Vorticella convallaria	1		1									1				
Number of species	7	6	6	6	6	6	7	6	3	3	2	8	8	6	5	4

[1]Abundances rated according to the scale given in Materials and Methods.

tertiary water treatment works are very different from those reported by Moravcova (1986) in quaternary groundwater wells of Czechoslovakia, where *Cyclidium* sp., *Mesodinium* sp., *Microthorax sp., Paramecium* sp., and *Spirostomum* sp. are most frequent. They are also different from the communities found by Stiller (1937), Török (1954), and Vejdovský (1882) in polluted springs and wells, and they differ from the communities reported by Verschaffelt (1929), Lloyd (1973), and Curds (1979) in slow sand filters, where *Tachysoma pellionellum, Oxytricha* sp., *Aspidisca cicada, Vorticella convallaria, Cyclidium* sp., and *C. heptatrichium* are most numerous[1]. However, Dornedden (1930) observed a protozoan community similar to that of the present study in slow sand filters near Berlin, viz. *Colpidium colpoda, Glaucoma scintillans, Stylonychia* spp., *Litonotus fasciola,* and various testate amoebae.

About 24 of the 38 species encountered feed on bacteria or are omnivores (Table 3), which is in line with the copious supply of bacteria in the biofilm. Most other species are predators which obviously benefit from the high productivity of the bacteria feeders. Most species found in the oxidators and filters are euryoecious, common inhabitants of various limnetic biotopes (Foissner et al., 1991, 1992, 1994, 1995). The most remarkable exception is *Loxophyllum utriculariae,* which was conspicuously numerous in some samples but has never been reported from potable water supplies or sewage-treatment processes and is also rare in other biotopes (Foissner et al., 1995). This rather large (up to 200 μm) pleurostome feeds on other ciliates and prefers clean water, which might explain its rather high abundance in the waterworks. A related species, *Loxophyllum meleagris,* has been found in slow sand filters (Curds, 1979).

The low diversity of the ciliates (5–13 species per sample) in the biofilm of the oxidators and filters is very likely caused by the monotonous structure of the biotope (gravel of defined size) and the food resources (single type of nutrient-poor water), the restricted access for species to invade the purification system (the whole process is sealed from the environment), and specific abiotic conditions (very low carbon content and presence of H_2S in the raw water). The first factors mentioned are possibly decisive, especially the restricted invasion possibility. Colonization depends on organisms which enter the system directly via the groundwater or by random chance via contaminated filter material and/or transient contacts of the groundwater with impure surface water. Soil is of course also a potential source of species for groundwaters and water treatment works (Husmann, 1978). However, not a single specimen of a typical soil ciliate, especially of colpodids which are so common and characteristic for terrestrial biotopes (Foissner, 1987, 1993), was seen in the samples investigated.

On the Significance of Protozoa in Potable Water Treatment Plants

The oxidators and filters contain not only a high number of ciliates and small metazoans but also many heterotrophic flagellates and amoebae (Gierig, 1993). The same applies to slow sand filters (Dornedeen, 1930; Husmann, 1978; Kisskalt, 1917; Liebmann, 1958; Lloyd, 1973; Richards, 1974). It is thus reasonable to assume that these organisms influence the purification process to some extent. However, detailed investigations are not available. The data from waste water treatment processes (Curds, 1992) and on the ecology of protozoa in general (Fenchel, 1987) suggest that protozoa enhance the energy flux in the biofilm and decrease the concentration of dispersed bacteria by their feeding (filtration) activity. This suggestion is supported by experiments showing a higher number of bacteria in the filtrate of slow sand filters if protozoans were killed by toxic agents (Kisskalt, 1917). However, these and other similar experiments were criticized

[1]The literature on protozoa in the water industry is loaded with poor species identifications. For the present purpose two taxa must be addressed, viz. *Holosticha pullaster* and *Acineria uncinata.* The former species has probably often been misidentified or at least not separated from *Tachysoma pellionellum.* A main characteristic of *H. pullaster* is the location of the contractile vacuole in the posterior third of the body (Fig. 5), as opposed to *T. pellionellum* where it is in midbody (Fig. 9). This difference is easily recognized even at low magnification (100×) with a conventional light microscope. *Acineria uncinata* (Figs. 2, 6–8) is very likely that species which is quite frequently reported as *Trachelophyllum pusillum* in the water treatment literature (Curds, 1992). However, the original description of *T. pusillum* is so incomplete that it is best considered as a species indeterminata. Furthermore, *A. uncinata* is easily confused with *Litonotus alpestris,* which has a similar size and shape but only one macronuclear nodule. See Foissner et al. (1991, 1995) for detailed descriptions of the species mentioned.

since the chemicals used very likely also affected the bacteria (Kustermann, 1958).

Protozoan grazing might increase the filtration capacity of the filters and oxidators by keeping the biofilm thin and the pores between the filter grains open (Kustermann, 1958). Husmann (1978) even suggests that the groundwater organisms prevent aquifers from being clogged by infiltrating organic residues throughout geological ages. This unconventional hypothesis has been supported recently by Sinclair et al. (1993) and Novarino et al. (in press), whose data indicate that protists may in fact play an important role in the biodegradation of organic contaminants in aquifer sediments.

Ciliates in Potable Water Treatment Works: Relations to the Saprobic System

Ciliates play a major role in the saprobic system which is extensively described in Curds (1992), Foissner et al. (1994), Liebmann (1951), and Sládeček (1973). Briefly, the saprobic system evaluates the quality of flowing waters in terms of protozoan and metazoan indicator species. Four major zones of organic pollution are distinguished: polysaprobity (very heavily polluted), alpha-mesosaprobity (heavily polluted), beta-mesosaprobity (moderately polluted), and oligosaprobity (clean or only slightly polluted).

Curds (1992, p. 70) concluded this from the few data available on ciliates in slow sand filters: "Surprisingly the ciliates found tend to be those associated with alpha-mesosaprobic conditions. This is unexpected since the organic loading of a slow sand filter is generally rather low and it is not usual practice to abstract water from heavily polluted sources." This conclusion is fully confirmed by the present data (Table 3), and I was as puzzled as Curds was when I saw the first samples from the filter backwash sludge and recognized the high abundance of *Colpidium colpoda,* a very sensitive indicator of microaerobic (i.e., heavily polluted) polysaprobic water (for detailed review see Foissner et al., 1994).

Waterworks of the type investigated and slow sand filters represent some sort of flowing water, and thus it should be possible to evaluate their organic load with the saprobic system. Certainly, the raw water is oligosaprobic (i.e., contains very few decomposable materials). There are, however, some inorganic nutrients and salts (e.g., nitrate, calcium) which accumulate due to the high filtration rate and obviously provide the nutrition for the biofilm (Gierig, 1993). A closer examination of the biofilm in the oxidators and filters showed that it contained sulphur bacteria (e.g., *Thiothrix*), indicating that some hydrogen sulphide from the raw water entered the filters or that the biofilm itself produces a microaerobic environment. Be that as it may, this observation suggests that *Colpidium colpoda* and the other alpha- to polysaprobic ciliates found were in "the right place." Furthermore, the copious bacteria growth in the biofilm can provide even large bacteria feeders, like *C. colpoda,* with sufficient food.

Obviously, it is the microenvironment which determines the occurrence of certain species. This concept is often overlooked by river ecologists and in discussions on the background of the saprobic system. Thus, although criticized by Curds (1992, p. 32), one of the central suppositions of the saprobic system could be correct, viz. that "such a classification presupposes that the respective organisms are uniquely dependent, within relatively narrow limits, on the chemical composition of the water for their distribution and development in situ." Future research on the autecology of the organisms used as bioindicators in the saprobic system should study the physical and chemical conditions of the water in the microenvironments of the organisms rather than, as is usual, the conditions in the freely flowing water.

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